SYNOPSIS OF PH.D TOPIC

Entitled

Study of Present Apiculture status, honey bee Species Diversity and Conservation remedies in Latur district (MS) India

Submitted to

Swami Ramanand Teerth Marathwada University Nanded-431606, Maharashtra State, India

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DECEMBER 2024

Title of Ph.D. Work

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1. Introduction:

Honeybees are communal insects that exist in colonies with ecological, evolutionary, and economic importance, and they are referred to as superorganisms [1,2,3]. Although Turkey has the honeybee subspecies of the Caucasian (*Apis mellifera caucasica*), Syrian (*Apis mellifera syriaca*), Iranian (*Apis mellifera)*, which have adapted to different geographical and climatic conditions, it has also various ecotypes that are morphologically and genetically defined [3,4,5,6,7,]. Honeybees are flying insects, and close relatives of wasps and ants. They are found on every continent on earth, except for Antarctica. Bees of all varieties live on nectar and pollen. Without bees, pollination would be difficult and time consuming - it is estimated that one-third of the human food supply depends on insect pollination. Bees have a long, straw-like tongue called a proboscis that allows them to drink the nectar from deep within blossoms. Bees are also equipped with two wings, two antennae, and three segmented body parts (the head, the thorax, and the abdomen). Honeybees are social insects that live in colonies. The hive population consists of a single queen, a few hundred drones, and thousands of worker bees.Honeybees forage for nectar and pollen from flowering plants. They use the nectar collected to create our favorite sweet treat - honey! When carrying the nectar back to the hive, their bodies break down the complex sucrose of the nectar into two simple sugars, fructose and glucose. [2] Tucking it neatly into a honeycomb cell, the bees will then beat their wings furiously over top of this syrupy sweet liquid to fan out the moisture and thicken the substance. When it is complete, the bees will cap that cell with beeswax, sealing the perfected honey for consumption later on.

Worker Bees: Worker bees are the most familiar-looking member of the honeybee hive, as they make up about 99% of each colony´s population. Bees of all varieties live on nectar and pollen. Without bees, pollination would be difficult and time consuming - it is

estimated that one-third of the human food supply depends on insect pollination. Bees have a long, straw-like tongue called a proboscis that allows them to drink the nectar from deep within blossoms. Bees are also equipped with two wings, two antennae, and three segmented body parts (the head, the thorax, and the abdomen). Honeybees are social insects that live in colonies. The hive population consists of a single queen, a few hundred drones, and thousands of worker bees.Worker bees are all female, and they do almost everything for the hive. From birth to her death 45 days later, the worker bee is given different tasks to do during different stages of her life. Worker bees are responsible for everything from feeding the larvae (the baby bees), to tending to the queen, to cleaning the hive, to collecting food, to guarding the colony, to building honeycomb. The stinger of the worker bee is barbed, so when she is forced to defend herself or the hive, her stinger will become stuck in the skin of her victim. She is unable to pull it out, and dies when she inevitably tears herself away from the stuck stinger, leaving it behind with the venom sack still pumping venom into her victim. Consequently, honeybees are very gentle - they don´t want to die any more than you want to be stung. Be nice to them, and they'll be nice to you.

Drone Bees: Male bees are called drones. Their job is to mate with queens from other hives. If they do get the opportunity to mate, they die immediately afterwards. If they do not mate, they can live up to 90 days (that's twice as long as a worker bee!) You can identify drones in the hive by their big round bodies and large eyes. Drones are incapable of stinging. keeping these views into the mind present investigation have been designed considering following objectives.

iii) Objectives:

- To investigate and enlist the abundance Honey bee species from different localities of Latur.
- To investigate the population of colonies and causes of dispersal of honey bees in Latur.
- Study of Diversity by Simpson's Index.
- To tabulate the number of Bee species in Latur region in selected villages.
- To enlist the pesticides dangerous for Honey bees colonies population
- To recommend the use of eco-friendly pesticides for increasing the Honey bees colonies in Latur.

1.1: Study site:

The district Latur (18.4088° N, 76.5604° E) this district has an historical background. The King 'Amoghvarsha' of Rashtrakutas developed the Latur city, originally the native place of the Rashtrakutas. The Rashtrakutas who succeeded the Chalukyas of Badami in 753 A.D called themselves the residents of Lattalut. The Latur geographical location shows that it situated south-eastern part of the Maharashtra state. in latur we will choose different sample sites to collect

Why we choose this topic?

Ecological Importance

1. Pollination services: Honey bees (Apis mellifera) are primary pollinators of many crops, including fruits, vegetables, and nuts.
2. Ecosystem health: Honey bees contribute to the health of ecosystems by pollinating flowers and maintaining plant diversity.

Biodiversity Conservation

1. Genetic diversity: Honey bee populations exhibit genetic diversity, which is essential for their adaptation to changing environments and resistance to diseases.
2. Species conservation: Studying honey bee diversity can inform conservation efforts for other bee species and pollinators.

Food Security

1. Crop yields: Honey bee pollination is crucial for maintaining crop yields and ensuring global food security.
2. Nutritional diversity: By pollinating a diverse range of crops, honey bees contribute to nutritional diversity and human health.

Climate Change Mitigation

1. Adaptation to climate change: Studying honey bee diversity can provide insights into their adaptation to climate change and inform strategies for maintaining pollination services.
2. Ecosystem resilience: Honey bee diversity can contribute to ecosystem resilience and the ability of ecosystems to withstand climate change.

2: Review of Literature

National status: Study at India

The classification of honey bees (*Apis* spp.) is important because of their economical (source of honey and bee products), nutritional, and ecological role (the bees pollinate wide varieties of crops and wild vegetation). Currently, the genus *Apis* has 10 commonly identified species i.e. *A. andreniformis, A. binghami, A. cerana, A. dorsata, A. florea, A. koschevnikovi, A. laboriosa, A. mellifera, A. nuluensis,* and *A. nigrocincta* (Arias and Sheppard, 2005). Based on the limited difference on morphological characters some species have been questioned e.g., *A. laboriosa* does not have enough morphological characters to be distinguished from *A. dorsata* (Koeniger and Koeniger, 1991, Engel, 1999) but both these have markable difference in performance and ecosystem (McEvoy and Underwood, 1988, Kirchner et al., 1996, Otis, 1996). The distribution of western honey bee, *A. mellifera* is an allopatric and its original habitat is Europe, Africa, and central Asia while the distribution of all other species of the genus *Apis* is Asia and these species are sympatric in tropical regions (Arias and Sheppard, 2005).

By using various molecular marker techniques, the genetic variety and gene pool of insects may be studied better and questions on distinguishing species based on morphometric characters can be answered more clearly. Mitochondrial DNA, random amplified polymorphic DNA, microsatellites, expressed sequence tags, and amplified fragment length polymorphism markers are important molecular techniques which are being employed in insect ecological studies and have contributed significantly in understanding the genetic origin of insect variety (Behura, 2006). Bees are mostly classified through their morphological characters, but their genetic variations are observed through mitochondrial DNA gene segments (Branchiccela et al., 2014, Ostroverkhova et al., 2015), single nucleotide polymorphism (Chapman et al., 2016), and allozymes (Smith and Glenn, 1995) to differentiate the races between the species. The molecular analysis offers a compelling tactic to measure the extent of genetic difference between and among the honey bee species (Meemongkolkiat et al., 2019).

In molecular markers, the mitochondrial genes are commonly used in insects to analyze the extent of genetic diversity (Bouga et al., 2011) since ten times high average mutation rates are

present in the mitochondrial genome in comparison to the nuclear genome (Ballard and Whitlock, 2004) owing to nucleotide imbalance (Song et al., 2003) and the insignificant effectual population size linked to active haploid heritage among eukaryotic taxa (Neiman and Taylor, 2009). The polyandrous mating approach in honey bees and other eusocial insects shows to introduce vast substitution ratios in mitochondrial genes as compared to nuclear genes because organisms inherit their mitochondrial DNA solely from the maternal lineage (Meusel and Moritz, 1993).

In Pakistan, total four species of the genus *Apis* are found. Out of these four species, three are native i.e., *A. cerana, A. dorsata, A. florea* while *A. mellifera* is imported from Europe (Khan, 2020, Sajid et al., 2020, Shakeel et al., 2020). There is very scant literature on morphological and molecular studies on the Asian and the western honey bee species found in the country. Although some molecular studies of *A. mellifera* were conducted based on mitochondrial gene segment i.e., cytochrome *c* oxidase I (Rizwan et al., 2018) but no molecular data about other Asian honey bee species are present.

So, the study was designed to better explore the extent of the genetic difference and phylogenetic interactions among three kinds of honey bee species (*A. cerana*, *A. dorsata*, and *A. mellifera*) from Pakistan and comparing them with additional honey bee species around the globe using partial sequence data from two mitochondrial genes (COI and ND5). The partial sequences of COI and ND5 were successfully amplified from the Asian and the western honey bee species and the ND5 sequences of *A. cerana*, *A. dorsata*, and *A. mellifera* were made for the first time from Pakistan. The data will offer balancing evidence and adequate knowledge of the phylogenetic difference and the basis among the genus *Apis*.

2.2: international status: Study Abroad India

Rates of land-use change in primary forests are increasing worldwide, threatening biodiversity (Montero-Castaño & Vilà, 2012). As a result, secondary forests become alternative habitats and resource providers that promote a faunal diversity more characteristic of primary forest (Peters et al., 2013; Taki et al., 2013). In tropical forests, one of the most threatened groups of fauna interacting in both primary and secondary forests are the pollinators (Winfree, Bartomeus & Cariveau, 2011; Cariveau & Winfree,

2015), a fact that highlights the importance of also conserving secondary forests (Taki et al., 2013; Winfree, Bartomeus & Cariveau, 2011).

The conservation strategy in Guatemala for the past twenty-five years has been to preserve primary forest in situ (National Congress of Guatemala, 1989) by creating protected areas without management strategies to preserve the primary forest surroundings. As a result of this policy, the people in Guatemala are unaware of the role or ascribe little importance to secondary forest in terms of conserving biodiversity. In SLT in Sololá, Guatemala, secondary forest is also underestimated; here, the traditional and conventional coffee farmers focus their conservation efforts on the pristine forest, mainly to ensure ecosystem services such as pollination and water provision. This is a good strategy for the conservation of native pollinators who nest in this forest (Jha & Dick, 2010; Klein, Dewenter & Tsccharntke, 2003; Klein et al., 2008; Rao & Stephen, 2010; Ricketts, 2004; Ricketts et al., 2008). However, many native pollinators also require secondary forest to obtain food resources throughout the year (Jha & Dick, 2010; Badano & Vergara, 2011; Klein et al., 2008; Kremen et al., 2004) and these are also important for maintaining biodiversity in general (Jules & Shahani, 2003; Kohler et al., 2008; Kremen et al., 2004; Kremen et al., 2007; Mandelik & Roll, 2009).

As some authors have suggested (Klein et al., 2007; Kremen, Williams & Thorp, 2002; Ollerton, Winfree & Tarrant, 2011; Winfree et al., 2007), among the vertebrate and invertebrate pollinators, bees are the most important pollination agents. Bees are responsible for pollinating nearly two thirds of crops worldwide (Brauman & Daily, 2008; Kremen, Williams & Thorp, 2002); however, climate change and habitat fragmentation have endangered bee diversity and reduced bee populations, leading to a food crisis worldwide. Conservation of pollinators has therefore emerged as an issue of great importance (Abrol et al., 2012; Ollerton, Winfree & Tarrant, 2011).

Around the world, two species of coffee dominate the world market, *Coffea canephora* L. y *Coffea arabica* L., the second being the one that dominates more than 60% of it (Ngo, Mojica & Packer, 2011; Enríquez et al. 2012). The global coffee market has suffered price fluctuations, however this has not meant that the cultivation has decreased (Ngo, Mojica & Packer, 2011). In recent years, Guatemala has become the seventh largest coffee producer in the world and it is the most important crop in the country in terms of the

employment and the foreign exchange that it produces (Asociación Nacional del Café (ANACAFE), 2014). Sololá is a high-quality coffee region and crop fields are gaining territory at the expense of the forest due to the high demand for coffee produced in the region (Asociación Nacional del Café (ANACAFE), 2014; Fischer & Victor, 2014).

At present in SLT, the secondary vegetation commonly known as "monte" is normally cut down or treated with herbicides to prevent the secondary growth. Conventional farmers argue that by keeping the surroundings of the coffee clean they reduce the incidence of coffee pests, although there is no scientific evidence to support this belief. They also believe that by maintaining some primary forests they can guarantee pollinator diversity. On the other hand, the indigenous people who practice traditional farming are aware of the importance of secondary vegetation (G. Armas-Quiñonez, 2014, personal observation). Through their traditional knowledge, they know that these represent a habitat for numerous important species. However, they also cut down all the secondary growth in common areas, arguing that it is for the safety of the children and for aesthetic purposes. In both cases, the secondary vegetation is removed. As a consequence, the area with secondary vegetation in the region could be insufficient to maintain the community of native bee pollinators, especially when the coffee is not in flower. In other words, the traditional and conventional coffee farmers are not aware of the critical importance of secondary forest to the preservation of the pollinators, a situation that must be addressed in Guatemala since it could lead to loss of biodiversity and subsequently to deficient coffee production (Philpott et al., 2008; Scheper et al., 2013; Steffan-Dewenter & Westphal, 2008).

In order to ensure production and obtain other income sources, coffee farmers of the Guatemalan highlands have introduced *Apis mellifera* hives into their farms. It has been shown that coffee is mainly pollinated by the honey bee but also frequently visited by stingless bees (Ngo, Mojica & Packer, 2011). However, the raising honey bees within coffee farms is practice may present some risk since it could have unknown impacts on native bee populations (Badano & Vergara, 2011; Garibaldi et al., 2011; Shavit, Dafni & Ne'eman, 2009; Van Engelsdorp & Meixner, 2010; Winfree et al., 2007), particularly in Guatemala where such interactions are poorly studied.

Coffee farming in Guatemala is very heterogeneous in terms of farming techniques. Big farms have changed from traditional management to conventional and highly intensive farming techniques that usually include higher inputs of agrochemicals, mainly pesticides, or on rare occasions have changed to integrated pest management. At the same time, traditional farmers use multi-farming techniques, where small coffee plantations are cultivated using intercropping with several banana hybrids (*Musa × paradisiaca*), papaya (*Carica papaya*), macuy *(Solanum americanum*), besides other crops. Traditional farmers usually cannot afford agrochemicals (pesticides, herbicides and fertilizers) to spray on their fields, and this management could therefore be contributing more than conventional coffee cultivation to the maintenance of bee diversity (Schmitt, 2006; Schüepp, Rittiner & Entling, 2012). However, in Guatemala, there have been no studies published that address this issue.

3. Materials and Methods:

Materials

1. Bee traps: Pitfall traps, pan traps, or Malaise traps to collect bee specimens.

2. Netting equipment: Insect nets to collect bees from flowers or nesting sites.

3. Sampling quadrats: Frames or plots to sample vegetation and bee diversity.

4. Camera traps: Camera traps to monitor bee activity and diversity.

5. DNA sampling kits: Kits to collect and preserve bee DNA samples.

6. Microscopes and dissecting equipment: For examining bee morphology and anatomy.

7. Statistical software: Software such as R, SAS, or SPSS for data analysis.

Methods

1. Field observations: Observing bee behavior, activity patterns, and interactions with flowers and other bees.

2. Bee surveys: Conducting systematic surveys to record bee species, abundance, and distribution.

3. Molecular analysis: Analyzing bee DNA to study genetic diversity, phylogeny, and population structure.

4. Morphometric analysis: Measuring bee body parts to study morphological diversity and variation.

5. Nesting site surveys: Surveying nesting sites to study bee nesting behavior, habitat preferences, and colony dynamics.

6. Pollinator effectiveness surveys: Assessing the effectiveness of different bee species as pollinators of various crops.

7. Experiments and manipulations: Conducting experiments to study bee behavior, ecology, and responses to environmental changes.

Sampling Techniques

1. Random sampling: Randomly selecting sampling locations or plots.

2. Stratified sampling: Dividing the study area into strata based on habitat or environmental characteristics.

3. Systematic sampling: Sampling at regular intervals or points.

4. Adaptive sampling: Adjusting the sampling design based on preliminary data or observations.

Data Analysis

1. Descriptive statistics: Calculating means, medians, and other summary statistics to describe bee diversity and abundance.

2. Inferential statistics: Using statistical tests to compare bee diversity and abundance among different habitats, regions, or time periods.

3. Multivariate analysis: Using techniques such as principal component analysis (PCA) or non-metric multidimensional scaling (NMDS) to analyze complex relationships among bee species and environmental variables.

4. Machine learning algorithms: Using algorithms such as random forests or support vector machines (SVMs) to classify bee species or predict bee diversity based on environmental characteristics.

Year wise Plan of work and targets to be achieve.

FIRST YEAR

- The identification of Bees species from Latur region will be done.
- List of 100 villages will be finalized to study.
- The list of pesticides affecting on honey bee species will be prepared from the study area.

SECOND YEAR

- Honey bee colony Population density and its relation with crop production will be studied.
- Causes for Honey bees population mortality and decrease will be searched and finalized
- Remedies to increase the honey bees colonies will be enlisted.

THIRD YEAR:

- All statistical calculation (mean and standard deviation) and graph making, thesis writing and submission process

Future perspective:

1. Assessing the vulnerability of honey bee species to climate change: Identifying which honey bee species are most vulnerable to climate change and developing strategies for their conservation.
2. Making Database for Future Researchers.

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